Application No.: 10/044,486 PATENT
Applicant: Tsien and Rao Attorney Docket No.: REGEN1510-1

Filed: January 11, 2002

Page 2

IN THE CLAIMS

1. (Currently amended) A compound having the general formula:

$$R$$
 A
 CO_2R'
 CO_2R'

in which R is a benzyl, 2-thienylmethyl, or cyanomethyl group; R' is selected from the group consisting of H, <u>alkyl</u>, physiologically acceptable salts or metal, <u>ester groups</u>, ammonium cations, --CHR₂OCO(CH₂)_nCH₃, --CHR₂OCOC(CH₃)₃, in which R₂ is selected from the group consisting of H and lower alkyl, acylthiomethyl, acyloxy-alphabenzyl, deltabutyrolactonyl, methoxycarbonyloxymethyl, phenyl, methylsulphinylmethyl, β-morpholinoethyl, dialkylaminoethyl, and dialkylaminocarbonyloxymethyl, and n is from 1-4; A is selected from the group consisting of S, O, SO, SO₂ and CH₂; and Z is a donor fluorescent moiety.

2. (Currently amended) The compound of claim 1, wherein the donor fluorescent moiety is selected from the group consisting of:

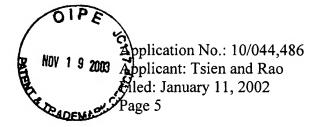
PATENT Attorney Docket No.: REGEN1510-1

$$R_3$$
 CO_2R_3
 F
 (VI)

PATENT Attorney Docket No.: REGEN1510-1

Application No.: 10/044,486
NOV 1.9 2003 Applicant: Tsien and Rao
Elled: January 11, 2002
Page 4

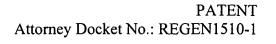
$$R_3$$
 S
 CO_2R'
 $(VIII)$



$$R_3$$
 C
 CO_2R'
 CO_2R'

$$R_3$$
 (IX)
 R_3
 (IX)
 R_3
 (IX)

(X)



Application No.: 10/044,486

MOV 1 9 2003 Applicant: Tsien and Rao

Gled: January 11, 2002

Frage 6

wherein R and R' are as defined in claim 1, R₃ is a linker for the fluorescent donor, X is H, F, Cl, Br, or CO₂R', or lower alkyl, and Y is N, CH, C-CN, or C-CF₃ or O.

3. (Currently amended) The compound of claim 2, wherein the linker is selected from the group consisting of a direct bond to a heteroatom in the fluorescent moiety, -- $O(CH_2)_n$ --, -- $S(CH_2)_n$ --, -- N^+R_2 (CH_2)_n, -- $OCONR_2$ (CH_2)_n--, -- $OCONR_2$

in which R_2 is as previously defined; and m and n are each independently integers from $[0] \underline{1}$ to 4.

Application No.: 10/044,486

NOV 1 9 2003 Applicant: Tsien and Rao

Fried: January 11, 2002

Age 7

4. (Currently amended) A The compound of claim 1, wherein the compound has having the structure:

5. (Withdrawn) A method for detecting the presence of β -lactamase activity in a sample, comprising:

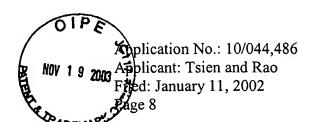
contacting the sample with at least one compound of general formula I:

$$R$$
 N
 CO_2R'
 CO_2R'

in which R is a benzyl, 2-thienylmethyl, or cyanomethyl group, or a quencher; R' is selected from the group consisting of H, physiologically acceptable salts or metal, ester groups, ammonium cations, --CHR₂OCO(CH₂)_nCH₃, --CHR₂OCOC(CH₃)₃, in which R₂ is selected from the group consisting of H and lower alkyl, acylthiomethyl, acyloxy-alpha-benzyl, deltabutyrolactonyl, methoxycarbonyloxymethyl, phenyl, methylsulphinylmethyl, β -morpholinoethyl, dialkylaminoethyl, and dialkylaminocarbonyloxymethyl; A is selected from the group consisting of S, O, SO, SO₂ and CH₂; and Z is a donor fluorescent moiety.

6. (Withdrawn) The method of claim 5, wherein said sample has a β -lactamase reporter gene.

PATENT Attorney Docket No.: REGEN1510-1



- 7. (Withdrawn) The method of claim 6, wherein said β -lactamase reporter gene is in a mammalian cell.
- 8. (Withdrawn) The method of claim 5, wherein samples having β -lactamase activity are separated from samples having no β -lactamase activity by fluorescent-activated cell sorting.
- 9. (Withdrawn) The method of claim 5, wherein the β -lactamase activity results from a β -lactamase enzyme that was prepared by mutagenesis of another β -lactamase enzyme.
- 10. (Withdrawn) The method of claim 5, wherein said compound is a membrane permeant derivative.
- 11. (Withdrawn) The method of claim 5, wherein the donor fluorescent moiety is selected from the group consisting of:

BAR RADEME

Attorney Docket No.: REGEN1510-1

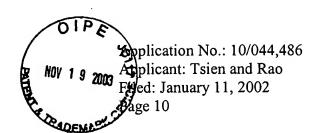
$$R_3$$
 X
 Y
 (III)

$$R_3$$
 O
 SO_3
 O_3S
 SO_3
 SO_3

$$R_3$$
 CO_2R_3 F (VI)

$$R_3$$
 N
 CO_2R'
 $(VIII)$

$$R_3$$
 X
 CO_2R'
 CO_2R'



R₃ is a linker for the fluorescent donor, X is H or lower alkyl, and Y is N or O.

12. (Withdrawn) The method of claim 11, wherein the linker is selected from the group consisting of a direct bond to a heteroatom in the fluorescent moiety, $--O(CH_2)_n$ --, $--S(CH_2)_n$ --, $--N^+R_2$ (CH₂)_n, $--OCONR_2$ (CH₂)_n--, $--O_2$ C(CH₂)_n--, $--SCSO(CH_2)_n$ --, $--SCSO(CH_2)_n$ --, $--SCSO(CH_2)_n$ --, $--SCSO(CH_2)_n$ --, $--SCSO(CH_2)_n$ --, $--SCSO(CH_2)_n$ --, $--SCSO(CH_2)_n$ --, and

$$-S \xrightarrow{\text{O} \\ \text{N(CH}_2)m} -$$

in which R₂ is as previously defined; and m and n are each_independently integers from 0 to 4.

13. (Withdrawn) The method of claim 5, wherein the compound has the structure:

- 14. (Withdrawn) A method for determining whether a compound of claim 1 is a substrate for a β -lactamase enzyme, comprising: contacting said compound with a sample containing said β -lactamase enzyme; exciting at the wavelength for the said compound when cleaved; and measuring fluorescence.
- 15. (Withdrawn) The method of claim 14, wherein said compound is a membrane permeant derivative.

Application No.: 10/044,486 PATENT
Applicant: Tsien and Rao Attorney Docket No.: REGEN1510-1

Filed: January 11, 2002

Page 11

16. (Withdrawn) The method of claim 14, wherein said β -lactamase enzyme has been prepared by mutagenesis of another β -lactamase enzyme.